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(54) Title: SYNTHESIS OF ENCODED POLYMERS

solid support

- chemical functionality
- "binding" strand
- novel building blocks

- information storage
- "coding" strand
- DNA or peptide
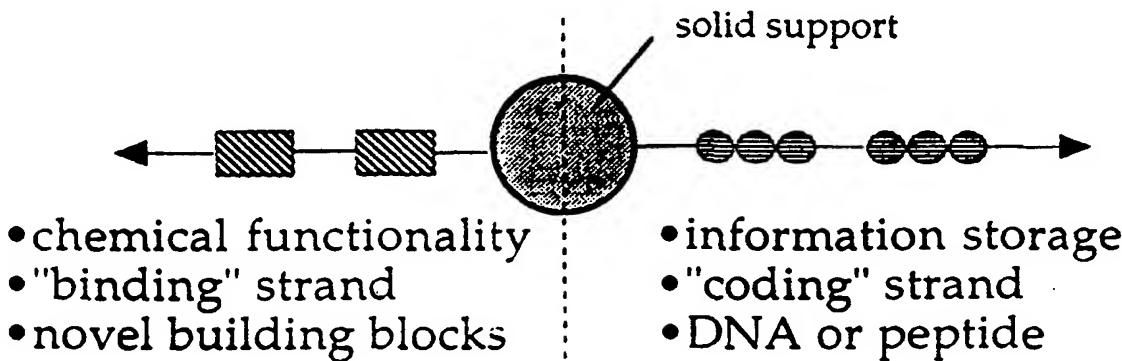
(57) Abstract

Conjugates and methods of producing the conjugates and mixtures thereof are disclosed. Conjugates are comprised of an active polymer made up of monomer units selected from the group consisting of the monomer units of peptides and/or peptoids, and an encoding polymer comprised of encoding monomers wherein the encoding polymer corresponds to the active polymer, and a coupling moiety covalently coupled to the active polymer and the encoding polymer. In accordance with the synthesis methodology, mixtures of large numbers of conjugates are produced by providing a coupling moiety and covalent binding it to an active monomer and an encoding monomer. Additional monomer units are added to the active monomer to create an active polymer and additional monomer units are added to the encoding monomer to produce an encoding polymer until the desired length is reached for the active polymer. Mixtures of conjugates attached to support bases can be used to assay a sample. The sample is brought into contact with the conjugates and a determination is made with respect to which active proteins bind to receptor sites within the sample. When active binding proteins are determined, the encoding polymer associated with the active polymer is sequenced, and by deduction, the sequence of the active polymer is determined.

5   SYNTHESIS OF ENCODED POLYMERS

Field of the Invention
          This invention relates to the fields of
biopolymer synthesis and drug design. More
10   particularly, the invention relates to methods for
synthesizing libraries of biologically active polymers
in association with an included polymer which is
encoded to facilitate deciphering.

15   Background of the Invention
          Modern pharmaceutical technology has taken
two divergent paths in pursuit of new therapeutic
compounds. Rational drug design achieves results by
intensive analysis of the molecular structure of
20   binding sites, and designing compounds specifically to
complement a desired binding site. For example, one
interested in preparing new antihypertensive compounds
might analyze the molecular structure of the β-
adrenergic receptor binding site using X-ray
25   crystallography and/or advanced NMR techniques, and
then synthesize compounds calculated to fit within the
binding site and complement the charge distribution.
          The other approach is to prepare an enormous
library of compounds and select only those compounds
30   which exhibit a desired activity. This approach
differs from the traditional pharmaceutical cycle of
design/synthesize/test/synthesize variants by
conducting the screening step in a massively parallel
fashion, screening an enormous number of different
35   compounds simultaneously. The challenge to this

approach is first to provide a group of compounds for
screening that is sufficiently numerous and diverse to
insure that the activity sought is represented in the
group, and second to identify the active compounds at

5   low concentration within the group.

Rutter et al., US 5,010,175 disclosed a
method of making diverse mixtures of peptides by
adjusting the concentration of each activated peptide
in proportion to its reaction rate, in order to obtain

10   a substantially equimolar mixture of peptides. Rutter
also disclosed the process of providing a mixture of
peptides (having at least 50 different peptides), and
selecting one or more peptides having a desired
property and separating them from the rest of the

15   peptides.

Zuckermann et al., PCT WO91/17823 disclosed
an alternative method for preparing diverse mixtures
of oligopeptides on solid-phase resins, and a robotic
device for performing the necessary manipulations. In

20   this method, a pool of resin particles is separated
into a number of groups (wherein each group is defined
as one or more separate reactions), and a different
amino acid coupled to the resin in each group. The
groups are then mixed together, separated into a

25   number of groups, and again coupled with a different
amino acid for each group. This cycle is repeated
until the desired number of amino acids per
oligopeptide is obtained. One advantage of this
approach is that each coupling reaction occurs in

30   isolation from other reactants, which permits one to
drive each reaction to completion without carefully
adjusting the initial concentration of each reactant.
This method also facilitates the preparation of
oligopeptides wherein some positions within the

35   peptide chain are held constant, and where some posi-
tions are restricted to less than all amino acids.
For example, one may use this method to prepare a pep-
tide of the formula $X_1-X_2-X_3-Glu-Ala-X_4-X_5-X_6$, where X_n

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can be any amino acid. If desired, one could limit,
for example, X_3 and X_5 to hydrophobic residues.
Zuckermann also disclosed that this method may be
applied to the synthesis of oligonucleotides, which
5   may then be inserted into cloning and expression
vectors for biological expression.

        Bartlett et al., PCT WO91/19735 disclosed a
variation of the Zuckermann et al. method in which a
diverse set of non-amino acid monomers is employed to
10   form mixtures of compounds called "peptoids."
Peptoids sample a different region of physico-chemical
parameter space than traditional oligopeptides,
depending on the type of linkage between monomers, and
may be able to exhibit activities unavailable to
15   peptide libraries due to the diversity (or difference)
in side chains.

        Houghten, US 4,631,211 disclosed a "tea-bag"
peptide synthesis method. The "tea bags" are mesh
bags containing resin beads for peptide synthesis.
20   Houghten's method enables one to add the same amino
acid to a number of different oligopeptides without
mixing the products: a number of "tea bags" may be
reacted with an amino acid in a common pot, then
separated physically.

25   Cook, EP 383620 described synthesis of COP-
1, a random polymer of Ala, Glu, Lys, and Tyr, having
an average molecular weight of 23 kDa having activity
in the treatment of multiple sclerosis. COP-1 is made
in the prior art by chemical polymerization of the
30   amino acids. However, Cook described expression from
genes made by random polymerization of
oligonucleotides, and selection for those clones
expressing COP-1 with the highest activity.

        Lebl et al., EP 445915 described a machine
35   for performing multiple simultaneous peptide syntheses
using a planar support surface. The planar support
is, for example, paper or cotton.

Kauffman et al., WO86/05803 disclosed production of peptide libraries by expression from synthetic genes which are partially or wholly "stochastic." Stochastic genes are prepared by polym-
5　erizing a mixture of at least three oligonucleotides (at least heptamers) to form a double-stranded stochastic sequence, and ligating the stochastic sequence into an expression vector.

Lam et al., WO92/00091 disclosed libraries
10　of oligonucleotides, oligopeptides, and peptide/nucleotide chimeras, and methods for screening the libraries for active compounds. However, Lam did not disclose conjugates having an active sequence and a coding sequence.

15　K.M. Derbyshire et al., Gene (1986) 46:145-52 disclosed a method for "saturation mutagenesis" of a segment of DNA, by synthesizing oligonucleotides using contaminated pools of monomer. Each A, C, G, and T reservoir contained 1/54 parts of each of the
20　other bases. The object was to prepare a DNA segment mixture having one or two mutations per sequence. They did not observe equal frequencies of mutation, presumably due to differences in coupling efficiency. The authors suggested synthesizing sequences using
25　four reservoirs containing pure bases, and one reservoir containing a mixture of all four bases in the concentrations necessary to balance the coupling efficiencies.

J.F. Reidhaar-Olson et al., Science (1988)
30　241:53-57 disclosed the generation of mutant λ repressor proteins by replacing two codons with random nucleotides (NNG/c). The resulting mutant proteins were assayed for activity to determine which amino acid positions were critical, and which positions
35　should be conserved.

I.S. Dunn et al., Prot Eng (1988) 2:283-91 disclosed the use of random polynucleotides to generate mutant β-lactamase α-peptides, some of which

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exhibited properties superior to the native sequence
α-peptide.

A.R. Oliphant & K. Struhl, <u>Nuc Acids Res</u>
(1988) <u>16</u>:7673-83 disclosed the use of random poly-
nucleotides to investigate promoter function. A
section of random polynucleotide was inserted into the
-35 to -10 region of a gene conferring drug resistance
in E. coli, and the transformants screened for resis-
tance. Survivors were cloned and sequenced to provide
a functional consensus sequence.

F.W. Studier, <u>Proc Natl Acad Sci USA</u> (1989)
<u>86</u>:6917-21 disclosed a method for sequencing large
volumes of DNA by random priming of cosmid libraries.

A.R. Oliphant et al., <u>Proc Natl Acad Sci USA</u>
(1989) <u>86</u>:9094-98 disclosed the generation of β-
lactamase mutants having altered properties, by
cloning a random polynucleotide into the β-lactamase
gene.

D.K. Dube et al., <u>Biochem</u> (1989) <u>28</u>:5703-07
disclosed the generation of β-lactamase mutants having
altered properties, by cloning a random polynucleotide
into the β-lactamase gene.

R.A. Owens et al., <u>Biochem Biophys Res Comm</u>
(1991) <u>181</u>:402-08 disclosed the selection of an HIV
protease inhibitor from a library of 240,000 tetrapep-
tides (in 22 mixtures). The mixtures were prepared by
the "mixed resin" technique.

These techniques enable one to prepare
libraries of diverse compounds. However, the problem
of identifying the resulting compounds has seldom been
addressed. Oligopeptides are typically sequenced by
stepwise cleavage of each amino acid from the parent
compound (which is usually immobilized on a resin),
with chromatographic analysis of the cleaved moiety.
Sensitive techniques are required to distinguish
between twenty or more amino acids. Analysis is
further complicated when uncommon amino acids are

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employed (using current techniques), especially when monomers are linked without using amide bonds.

Summary of the Invention

The present invention provides a method of synthesizing true mixtures of diverse oligopeptides and/or peptide-like compounds along with an associated encoding polymer making it possible to easily analyze those compounds exhibiting a desired activity. The invention involves synthesizing an encoding DNA strand simultaneously with the peptide/peptoid. Each unique peptide/peptoid sequence associated with its own unique DNA strand to provide the conjugates of the invention. These conjugates are screened to determine which peptide/
peptoid compounds exhibit a desired activity, and the active conjugates analyzed by DNA sequencing methods to determine the attached peptide/peptoid sequence by deduc-tion, i.e., since each DNA sequence is associated with a known peptide/peptoid, once the DNA sequence is deter-
mined, the sequence of the peptide/peptoid can be deduced.

Another aspect of the invention is a conjugate comprising a peptide or peptoid coupled to and/or directly associated with a coding polymer (CP), e.g. a nucleic acid (NA). The peptide/peptoid/CP conjugate may be linked directly (i.e., covalently bound either directly or through a small organic mol-ecule), or by linkage to the same support (e.g., by synthesizing both peptide/peptoid and CP strand on the same particle or bead of resin).

An important object of the invention is to provide a chemical synthesis method which allows the production of libraries of peptides and/or peptoids along with a unique encoded polymer such as a DNA strand which makes it possible to readily determine the sequence of the peptide or peptoid.

An advantage of the present invention is that the methodology makes it possible to readily identify and sequence peptides and/or peptoids having desirable biological activities.

5   A feature of the present invention is that sequences of peptoids or peptides which contain nonconventional amino acids can still be readily determined by sequencing associated polymers such as DNA sequences which are simultaneously synthesized

10   with the peptoids and encode them.

These and other objects, advantages and features of the present invention will become apparent to those persons skilled in the art upon reading the details of the structure, synthesis and use as more

15   fully set forth below, reference being made to the accompanying figures forming a part hereof.

Brief Description of the Drawings

Figure 1 is a schematic diagram showing a

20   specific embodiment of a conjugate of the invention which conjugate includes a "binding" strand or active polymer attached to a solid-support substrate which substrate is also bound to an information storage or "coding" strand;

25   Figure 2 is a schematic flow diagram demonstrating how encoded libraries can be synthesized on beads as the solid-support substrate;

Figure 3 is a schematic diagram showing methods of the synthesis of both solid-phase and

30   solution-phase libraries;

Figure 4 is a schematic diagram showing resin-bound libraries generated by the derivatization of non-hydrolyzable resins;

Figure 5 is an HPLC chromatogram of binding

35   and coding peptide strands simultaneously synthesized via non-hydrolyzable resin linkage;

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Figure 6 is an HPLC chromatogram of a coding and binding strand adduct which was synthesized via a hydrolyzable resin linker;

Figure 7 is a plotted graph resulting from ELISA competition of binding sequences versus binding/encoding sequences;

Figure 8 is a schematic diagram showing the analysis of a solid-phase amptide; and

Figure 9 is a schematic flow diagram showing the analysis of a solution-phase amptide.

Detailed Description of Preferred Embodiments

Before the present method of synthesis, conjugates and methods of using such are described, it is to be understood that this invention is not limited to the particular methodologies, conjugates, or methods of use described as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting since the scope of the present invention will be limited only by the appended claims.

It must be noted that as used herein and in the appended claims, the singular forms "a," "and," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a peptide" includes mixtures of peptides, reference to "an amino acid" includes mixtures thereof, and reference to "the reaction" includes one or more reactions of the same type as generally understood by those skilled in the art, and so forth.

Unless defined all otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or

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testing of the present invention, the preferred
methods and materials are now described. All
publications mentioned herein are incorporated herein
by reference to describe and disclose specific
5   information for which the reference was cited in
connection with.

In general, the invention provides a rapid
method of synthesizing large numbers of conjugates
which conjugates are comprised of a peptide/peptoid
10   sequence, e.g., an amino acid sequence associated with
a unique encoding sequence, e.g., a DNA sequence. The
conjugates can be readily synthesized and thereafter
screened for biological activity, and when activity is
found, the particular peptide/peptoid sequence found
15   to be active can be readily identified by its
associated encoding (DNA) strand. Each conjugate of
the invention is comprised of at least two components
with one of the components being the peptide or
peptoid sequence which binds to a receptor of interest
20   and the other sequence being a polymer which encodes
the binding sequence. The invention may utilize
standard amino acids and DNA as encoding monomers to
produce a chemically diverse library of solution-phase
or solid-phase conjugates. In order to further
25   describe the invention in detail, the following
definitions are provided.

A.   Definitions

The terms "nucleic acid" and "NA" refer to
30   oligomers constructed from DNA and/or RNA bases which
may be sequenced using standard DNA sequencing tech-
niques. The NAs used herein may include uncommon
bases so long as such bases are distinguishable from
the other bases employed under the DNA sequencing
35   methods to be used and include peptide-nucleic acids
(PNAs) (disclosed by Nielsen, P.E., Egholm, M., Berg,
R.H. & Buchardt, O., Science (1991) 254, 1497-1500).
Such PNAs could serve as coding strands and the

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detection would be by hybridi-zation. NAs will
usually be constructed from monomers linked by phos-
phodiester bonds, but other similar linkages may be
substituted if desired. For example,
5   phosphorothioates may be employed to reduce lability.
The term "peptide" as used herein refers to
the 20 commonly-occurring amino acids: alanine (A),
cysteine (C), aspartic acid (D), glutamic acid (E),
phenylalanine (F), glycine (G), histidine (H),
10   isoleucine (I), lysine (K), leucine (L), methionine
(M), asparagine (N), proline (P), glutamine (Q),
arginine (R), serine (S), threonine (T), valine (V),
tryptophan (W), and tyrosine (Y).
The term "peptoid" as used herein refers to
15   a non-peptide monomer of the general formula $(R)_n$-X-
$(L)_m$, where R is a side chain group, n is at least 1,
L is a linking group, m is at least 2, and X is a
small organic radical. It is preferred to select L
radicals that may be individually protected and
20   deprotected. Preferably n will be 1 or 2 and m will
be 2. Monomers wherein m is 3 or greater may be used
to form branched active polymers. Presently preferred
monomers are N-substituted glycine derivatives of the
formula

25

$$
HN-CH_2-C(=O)-\left[\begin{array}{c}R\\ |\\ N\\ |\\ R\end{array}-CH_2-C(=O)\right]_n-\overset{R}{\underset{|}{N}}-CH_2-C(=O)-X
$$
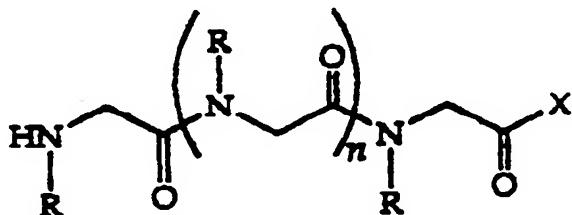
30   wherein R is alkyl of 2-6 carbon atoms, haloalkyl of
1-6 carbon atoms wherein halo is F, Cl, Br, or I,
alkenyl of 2-6 carbon atoms, alkynyl of 2-6 carbon
atoms, cyclolkyl of 3-8 carbon atoms, alkoxyalkyl of
2-8 carbon atoms, aryl of 6-10 carbon atoms, arylalkyl
35   of 7-12 carbon atoms, arylalkyl of 7-12 carbon atoms
substituted with 1-3 radicals independently selected
from halo and nitro and hydroxy, aminoalkyl of 1-6
carbon atoms, hydroxyalkyl of 1-6 carbon atoms,

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carboxy, carboxyalkyl of 2-6 carbon atoms,
carboalkoxy-alkyl of 3-10 carbon atoms, carbamyl,
carbamylalkyl of 2-6 carbon atoms, imidazolyl, imid-
azolylalkyl of 4-10 carbon atoms, pyridyl,
5   pyridylalkyl of 6-10 carbon atoms, piperidyl, pip-
eridylalkyl of 5-10 carbon atoms, indolyl, or
indolyalkyl of 9-15 carbon atoms. Thus, active
polymers composed of these monomers are equivalent to
polyglycine having side chains attached at each
10   nitrogen. These and other monomers are described in
copending application USSN 07/715,823, incorporated
herein by reference, and PCT WO91/19735.

The terms "coding" and "encoding" indicated
that one or more coding monomers corresponds directly
15   and uniquely to a given active monomer, e.g.,
conventional
nucleic acids encode (in groups of three) the 20 natural
amino
acids. The number of coding monomers used for each
20   code depends on the number of different coding mono-
mers and the number of different active monomers.
Typically, the number of different active monomers
used will range from about 5 to about 30. A basis set
of 4 coding monomers can encode up to 16 active
25   monomers taken in "codons" of 2 coding monomers. By
increasing the coding monomer basis set to five
distinct monomers, one can encode up to 25 different
peptide/peptoid monomers. A basis set of 4 coding
monomers can encode up to 64 peptide/peptoid monomers
30   taken in "codons" of 3 coding monomers. Note that one
can make the code degenerate or nondegenerate, and can
insert additional coding information into the
sequence. For example, one may wish to begin each
codon with the same base (e.g., G), using that base
35   only in the first position, thus unambiguously identi-
fying the beginning of each codon. As a practical
matter, the group of monomers selected for use as
coding monomers will form polymers that are easier to

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sequence than the active polymers, i.e., the coding
monomers may be more readily identified using present
day sequencing technology as compared to the monomer
of the active polymers. With current technology, the
5   order of preference for coding monomers is nucleic
acids > peptides > peptoids. Nucleic acids have the
additional advantage that the coding sequence may be
amplified by cloning or PCR (polymerase chain
reaction) methods known in the art.
10   The term "active polymer" and/or "binding
polymer" refers to a polymer having a desired
biological activity. Suitable biological activities
include binding to natural receptors, pharmaceutical
effects, immunogenicity/antigenicity, and the like.
15 "Immunogenicity" refers to the ability to stimulate an
immune response (whole or partial serum-mediated
immunity and/or cell-mediated immunity) in a bird or
mammal following administration. Antigenicity
requires only that the active polymer bind to the
20 antigen-binding site of an antibody. Pharmaceutical
activities, for the purposes of this invention, will
generally depend on the ability of the active polymer
to bind a protein, carbohydrate, lipid, nucleic acid,
or other compound present in the subject. For
25 example, an active polymer may bind to a cell surface
receptor and compete with the receptor's natural
ligand, with or without activation of the receptor.
Other useful pharmaceutical activities include
cleavage of endogenous molecules (e.g., protease
30 activity, nuclease activity, and the like), catalysis
of reactions either primarily or as a cofactor,
donation of functional groups (e.g., acyl, ATP, alkyl,
and the like), pore formation, and the like. Active
polymers comprise a series of monomers which are
35 linked sequentially. The monomers will generally be
peptides, peptoids, or carbohydrates in the practice
of the instant invention.

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The term "mixture" as used herein refers to a composition having a plurality of similar components in a single vessel.

The term "couple" as used herein refers to formation of a covalent bond.

The term "coupling moiety" refers to a soluble or insoluble support to which can be attached one or more active monomers and the corresponding encoding monomers. Insoluble supports ("solid support means") may be any solid or semi-solid surface which is stable to the reaction conditions required for synthesis of the active and coding polymers, and is suitable for covalently attaching and immobilizing both polymers, for example, most resins commonly employed in DNA and peptide synthesis, such as MBHA, Rink, and the like. The particular resin used will depend upon the choice of coding and active polymers and their associated synthetic chemistries. Soluble coupling moieties are molecules having functional groups to which active and coding monomers may be attached. Each soluble coupling moiety must be able to accommodate at least one coding polymer and at least one active polymer, although the active and coding polymers need not be present in a 1:1 ratio. The soluble coupling moiety may be as simple as an amino acid having an functional group in its side chain, or may be as complex as a functionalized (soluble) polymer.

The term "conjugate" as used herein refers to the combination of any "active polymer" and its associated "coding" polymer. The conjugate may be formed using a "coupling moiety" or by binding both the "active polymer" and "encoding polymer" to the same support surface in close proximity with each other so that the two polymers are "associated" with each other. When both polymers are bound to the same support surface, such as a small bead, the encoding polymer can be readily sequenced off of the bead and

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the other "active polymers" remaining on the bead will
be identified once the encoding sequence is known.

B.   Related Libraries and Synthesis Methodologies for
5   producing same.
            There are many limitations with the current
technologies for probing the receptor-binding
properties of peptide libraries.   Filamentous
bacteriophage libraries offer the largest source of
10   peptide diversity ($\approx 10^7-10^8$ different components) of
any current technology to date (Scott, J. & Smith, G.,
Science, (1990), 249, 386-390; Devlin, J., Panganiban,
L. & Devlin, P., Science, (1990), 249, 404-406;
Cwirla, S., Peters, E., Barret, R. & Dower, W., Proc.
15   Natl. Acad. Sci. U.S.A., (1990), 87, 6378-6382).
These libraries, however, are limited to the natural
set amino acids, suffer from biological biases (i.e.,
varying rates of growth, proteolysis, etc.) and also
suffer, in practice, from high levels of background
20   binding.   The present invention is designed to
overcome these difficulties.
            Multiple-peptide synthesis technology has
substantially increased the ability to generate
individual peptides (Geysen, H., Meloen, R. &
25   Barteling, S., Proc. Natl. Acad. Sci. U.S.A., (1984),
81, 3998-4002; Houghten, R., Proc. Natl. Acad. Sci.
U.S.A., (1984), 5131-5135; Schnorrenberg, G. &
Gerhardt, H., Tetrahedron, (1989), 45, 7759-7764;
Gausepohl, H., Kraft, M., Boulin, C. & Frank, R. in
30   Peptides: Chemistry, Structure and Biology
(Proceedings of the 11th American Peptide Symposium,
(1990), eds. Rivier J. & Marshall, G., (ESCOM,
Leiden), pp. 1003-1004; Frank, R. & Döring, R.,
Tetrahedron, (1988), 44, 6031-6040; Fodor, S., Read,
35   J., Pirrung, M., Stryer, L., Lu, A. & Solas, D.,
Science, (1991), 251, 767-773).   The synthesis of $\approx 10^4$
individual peptides per cm^2 of glass wafer represents
the diversity limit of this technology (Fodor, S.,

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Read, J., Pirrung, M., Stryer, L., Lu, A. & Solas, D.,
Science, (1991), 251, 767-773). A mixed-resin
algorithm method (Furka, A., Sebestyén, M., Asgedom M.
& Dibó, G., Int. J. Peptide Protein Res., (1991), 37,
5   487-493) has recently been used to generate solution-
phase libraries (Houghten, R., Pinilla, C., Blondelle,
S., Appel, J., Dooley, C. & Cuervo, J., Nature,
(1991), 354, 84-86) and resin-bound peptide libraries
(Lam, K., Salmon, S., Hersh, E., Hruby, V.,
10   Kazmiersky, W. & Knapp, R., Nature, (1991), 354, 82-
84) that contain $\approx 10^6$ and $\approx 10^7$ components,
respectively. The solution-phase libraries offer the
advantage of providing quantitative receptor-binding
information (Zuckermann, R., Kerr, J., Siani, M.,
15   Banville, S. & Santi, D.V., Proc. Natl. Acad. Sci.
U.S.A., 89, 4505-4509 (1992)). Furthermore, these
libraries allow the affinity of the solution
conformation of a ligand to be determined, a quantity
that is essential for rational drug design. An
20   apparatus for the automated synthesis of equimolar
peptide mixtures is disclosed in Zuckermann, R.N.,
Kerr, J.M., Siani, M.A. & Banville, S.C., Int. J. Pep.
Pro. Res., (1992), 40, 498-507.

The publications cited and discussed above
25   can be used in producing the active or binding polymer
which is used in producing the conjugate of the
present invention. Accordingly, the disclosures of
all of these publications are incorporated herein by
reference in order to disclose peptide and peptoid
30   synthesis methodology. Although the methodology
discussed within these references is extremely
valuable with respect to the production of large
amounts of different types of binding polymers, the
mixtures of polymers produced by this methodology are
35   often so large and complex that there are many
practical limitations with respect to their actual
analysis and use. The present invention can be
readily applied with such synthesis methodologies in

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order to provide an efficient, commercially practical
method of analyzing the proteins produced using such
methodology.

Both the mixed-resin and solution-phase
5   methods, however, do not allow incorporation of many
non-standard amino acids because of the limitations of
peptide analysis. Resin-bound peptide libraries, in
particular, suffer from a relatively slow rate of
analysis (peptide sequencing at 3 beads per day) and
10   are limited in complexity to $\approx 10^7$ beads/ml. In order
to generate a "complete" peptide library, there must
be multiple copies (>10) of any given peptide
sequence. This becomes problematic at the sequencing
stage because the same "hit" sequence may appear
15   multiple times. The alternative is to work with
libraries that are not complete at the risk of losing
sequences that bind.

When using the methodology of the present
invention, the sequence of a biologically active
20   protein can be determined even without isolating the
protein of interest. This can be done by synthesizing
large numbers of different proteins on large numbers
of different support surfaces such as small beads. An
encoding polymer is attached to beads to identify each
25   protein. A sample to be tested is then brought into
contact with the beads and the beads are observed with
respect to which proteins bind to a receptor site in
the sample. The bead having the receptor bound
thereon is analyzed by sequencing the coding polymer
30   which has also been synthesized on the bead. When the
encoding polymer has been sequenced, the sequence of
the active polymer, which may be a peptide, can be
readily deduced. Thus, the present invention makes it
possible to determine the activity and sequence an
35   active polymer, such as a biologically active peptide,
without ever isolating the peptide.

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C.   Underline: General Methodology

        This invention describes a methodology for
the synthesis and screening of large synthetic polymer
libraries that contain non-standard amino acids and
5   even non-amide based polymers. The strategy utilizes
a modified mixed-resin peptide synthesis methodology
to simultaneously synthesize two polymer sequences:
one polymer strand (the "binding" strand) is
synthesized for the intended purpose of receptor
10 binding, and the second strand (the "coding" strand)
contains standard amino acids or deoxyribonucleotides
that encodes for the binding strand (Figure 1). The
ability to decipher the binding sequence by analysis
of the coding strand with standard peptide or
15 oligonucleotide techniques allow the inclusion of a
wide variety of novel building blocks and
conformational constraints into a diverse ligand
library.

        This invention also describes a methodology
20 to increase the size ($>10^8$) and screening rate of a
ligand library. The method uses two polymers as
above, but specifically utilizes an
oligodeoxyribonucleotide for the "coding" strand. The
use of DNA as the coding strand allows for an
25 increased sensitivity of detection (fmol vs pmol for
peptide analysis). This increased sensitivity allows
for a larger library size since the amount of polymer
needed for detection is reduced dramatically. The
rate of sequence determination of receptor binders is
30 increased since many samples can be analyzed in
parallel.

        In order to couple a polymer's sequence
information with a peptide or oligonucleotide
sequence, there needs to be a method that
35 unambiguously correlates each polymer to each other.
Thus, when any particular non-standard amino acid (or
other monomer) is added to a "binding" polymer chain,
the corresponding information (amino acid or

-18-

nucleotide monomer) must also be added to the "coding" strand. A "genetic code" is thus established (Table 1) where each binding monomer corresponds to (a multiple) of standard amino acids or nucleotides on

5   the coding strand. For example, the use of three standard amino acids or nucleotides, in a 3:1 ratio with a novel monomer, would allow for the unambiguous representation of 27 novel monomers.

10   TABLE 1. Custom genetic code.

# of bases	"codon" length	# of monomers coded for
2	3	8
2	4	16
2	5	32
3	2	9
3	3	27
4	2	16
4	3	64

20

The synthesis of coded libraries requires a modified mixed-resin algorithm (Figure 2). The resin beads are divided into equal portions, a unique monomer is added to the "binding" strand, followed by

25 the coupling of a corresponding amino acid or nucleotide to the "coding" strand. The resin aliquots are then combined to generate a mixture. A set of compatible protecting groups is thus required to preferentially deprotect and extend each strand

30 independently.

Two synthesis formats are possible for amptide libraries, one that generates resin-bound libraries and one that generates solution-phase libraries (Figure 3). Resin-bound libraries can be

35 synthesized using non-hydrolyzable linkers that are derivatized with the "binding" and "coding" monomers strands. Solution-phase libraries can be synthesized

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as a 1:1 polymer:peptide/DNA conjugate via a
hydrolyzable linker attached to the resin.

Peptide as the "Coding" strand

5   The use of base-labile Fmoc-protected
monomers and acid-labile (N^α-Ddz-protected amino acids
(Birr, C., Nassal, M., Pipkorn, R., Int. J. Peptide
Protein Res., (1979), 13, 287-295), for example, allow
for selective deprotection and coupling to two
10   individual polymer strands. Resin-bound libraries can
be generated by the derivatization of non-hydrolyzable
resins with a 1:1 ratio (or any desired ratio) of
Fmoc:Ddz monomers (Figure 4). This introduces two
differently protected amino acids that an be extended
15   independently. Solution-phase libraries that contain
a 1:1 ratio of binding:coding strands can be
synthesized by using a hydrolyzable Fmoc-Lysine(Moz)-
OH linker that allowed for chain growth at both the α-
and ε-amino groups. Amino acids which do not contain
20   functional groups are preferred for the "coding"
strand in order to minimize unwanted binding
interactions.
          The receptor-binding ligand can be
identified by bead staining techniques (Lam, K.,
25   Salmon, S., Hersh, E., Hruby, V., Kazmiersky, W. &
Knapp, R., Nature, (1991), 354, 82-84) and the
sequence determined by N-terminal Edman degradation.
In order to ensure that only the "coding" strand is
sequenced, it is essential that the N-terminus of the
30   "binding" strand be acetylated or otherwise made non-
sequencable.

DNA as the "Coding" Strand

          The construction of libraries with DNA as
35   the coding strand is similar to those with peptides
but offers several advantages: the information
storage and replicative properties of DNA allow for

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increased sensitivity of detection, a larger library
size and an increased rate of sequence determination.

The synthesis of DNA as the coding polymer
requires compatibility between the assembly of Fmoc-
5   based monomers and standard DNA chemistry. These
synthesis strategies are likely to be compatible ((a)
Juby, C., Richardson, C. & Brousseau, R., Tet.
Letters, (1991), 32, 879-882. (b) Haralambidis, J.,
Duncan, L., Angus, B. & Tregear W., Nucleic Acid Res.,
10   (1990), 18, 493-499) (see Table 2). Alternatively,
allyl-based protection strategies exists for both
peptide (Lyttle, M.H.; Hudson, D., Peptides: Chemistry
and Biology (Proceedings of the 12th American Peptide
Symposium): Smith, J. and Rivier, J.E., Eds.; ESCOM,
15   Leiden, 1992, pp. 583-584) and
oligodeoxyribonucleotide (Hayakawa, Y., Wakabayashi,
S., Kato, H. & Noyori, R., J. Am. Chem. Soc., (1990),
112, 1691-1696) synthesis. The assay of solution-
phase libraries can be facilitated by using only
20   pyrimidines in the coding strand, thereby avoiding the
potential problem of base pairing between individual
strands.

Strategies for the synthesis of coding and
active polymers, as well as matching the active
25   polymer with a coding sequence to provide a genetic
tag are described in Brenner et al. Proc. Natl. Acad.
Sci. U.S.A., 89:5381-5383 (June 1992) which is
incorporated herein by reference.

30

35

TABLE 2

Compatibility of DNA vs. Peptide Synthesis Chemistry

Peptide chemistry	Oligonucleotide Chemistry
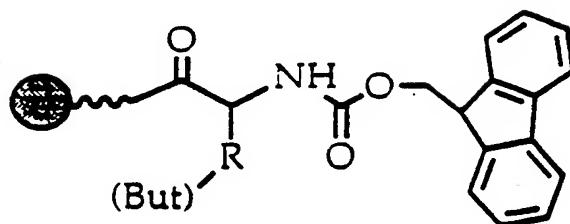
Permanent Protecting Groups:

-OH	t-butyl ether	A,C	benzoyl amide
-CO₂H	t-butyl ester	G	isobutyryl
-NH₂	t-boc	-P=O	cyanoethyl, methyl
his, cys	trityl		
arg	sulfonyl		

Note: -CO₂H rendered as $-CO_2H$, -NH₂ as $-NH_2$

removed by:

85% trifluoroacetic acid 2 hours @ room temp	conc. NH₄OH 55°C 5 hours

Temporary protecting groups:

9-fluorenylmethoxycarbonyl	4,4-dimethoxytrityl

removed by:

20% piperidine	3% trichloroacetic acid

Cleavage from solid support:

85% trifluoroacetic acid 2 hours @ room temp	conc. NH₄OH 55°C 5 hours

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TABLE 2 (Cont)

Compatibility of DNA vs. Peptide Synthesis Chemistry

Std. conditions	Alternative	Problem addressed
t-Butyl carbamate/ ester	allyl	amino acid deprotection from detritylation reagent
20% Piperidine	2% DBU	substitution at exocyclic amine
cyanoethyl phosphate	allyl	acylation of phosphate
NH₄OH	ethylenediamine/ EtOH	racemization
iodine	t-Butylhydro-peroxide	Tyr, Met, Cys oxidation
controlled pore glass	polystyrene resin	resin transferring
T, C & G	T, C, G & A	depurination
T, C	T, C, G & A	base pairing

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Resin-bound libraries can be synthesized by using non-hydrolyzable linkers to attach both the C-terminus of the peptide and the 3'-end of the oligonucleotide to the same bead. Solution-phase
5   libraries can be synthesized as a 1:1 peptide-oligonucleotide conjugate, in which the C-terminus of the peptide is attached to the 3'-end of the oligonucleotide through a hydrolyzable Fmoc-Ser(O-Dmt) linker which is attached to the resin.
10   The identification of binders in the resin-bound peptide libraries can be detected by the bead staining methodology (Lam, K., Salmon, S., Hersh, E., Hruby, V., Kazmiersky, W. & Knapp, R., _Nature_, (1991), 354, 82-84). Although the peptides are bound to a
15   solid-phase, there does not have to be a 1:1 peptide-oligonucleotide ratio since the DNA can be amplified prior to the determination of its sequence. In fact, less DNA is preferred so that there will be less interference with the polymer's binding properties.
20   Once a bead is identified, the DNA sequence is determined (Stahl, S., Hultman, T., Olsson, A., Mois T., et al., _Nucleic Acid Res._, (1988), 16, 3025-3038) after PCR amplification or by thermal-cycle sequencing (Figure 8). This requires the inclusion of one or
25   more primer sites neighboring the coding region of the oligonucleotide. Similarly, the use of solution-phase libraries requires isolation of each sequence from each other. This can be accomplished by restricting the DNA after PCR amplification and inserting it into
30   M13 (or other suitable vector) for clonal isolation and sequencing (Figure 9).

EXAMPLES

The following examples will provide those
35   skilled in the art with a complete disclosure of how to make and use the invention and are not intended to limit the scope of the invention. Efforts have been made to insure accuracy with respect to numbers used

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(e.g. amounts, temperature, etc.), but some
experimental error and deviation should be accounted
for. Unless indicated otherwise, parts or parts by
weight, molecular weight is weight average molecular
5   weight, temperature is in degrees centigrade and
pressure is at or near atmospheric.

Example 1

The independent synthesis of two
10   unambiguously correlated sequences has been
successfully completed. The subsequent sequence
analysis of the "coding" strand has also been
demonstrated. For convenience, two peptide sequences
were chosen. The "binding" strand was synthesized
15   with N^α-Fmoc-protected amino acids and the "coding"
strand was synthesized with N^α-Ddz-protected amino
acids. The simultaneous synthesis of the two peptide
strands was tested in two formats, 1) resin-bound
peptide library synthesis and 2) solution-phase
20   peptide libraries using a hydrolyzable Fmoc-Lys(Moz)-
OH linker (Wang, S.S.; Chen, S.T, Wang, K.T., and
Merrifield, R.B., Int. J. Peptide Protein Res.,
(1987), 30, 662-667). These syntheses were performed
on single peptides (not libraries) as a demonstration
25   of research concept and in order to allow the full
characterization of the synthesis products.

30

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A. Synthesis of a Resin-Bound Library Model

"Binding" Sequence: Ac-Arg-Leu-Val-Thr-His
(Fmoc peptide)

```
                                                    ╲
                                                     ╲ Resin
                                                     ╱
```

"Coding" Sequence: H$_2$N-Ala-Ser-Gly-Glu-Phe-Ala
(Ddz peptide)

Synthesis Scheme:

Step #	Description
1	Derivatization of MBHA resin with 1:1-Ddz-Ala-OH: Fmoc-His(Trt)-OH
2	Deprotection of "binding" strand with 20% piperidine/DMF
3	Coupling of second "binding" amino acid, Fmoc-Thr(But)-OH
4	Deprotection of "coding strand" with 7.5% TFA/CH$_2$Cl$_2$
5	Coupling of second "coding" amino acid, Ddz-Phe-OH
6	Repeat steps 2-5 with alternative Fmoc and Ddz deprotection and corresponding amino acid coupling
7	Final Deprotection of Fmoc followed by acetylation
8	TFA deprotection of side-chain groups

After the TFA deprotection, the model library bead has two independently synthesized sequences and is ready for assay. Only the coding strand has a free α-amino group and can be characterized by N-terminal Edman degradation. The binding strand is acetylated and there-fore will not interfere with the sequencing. The two peptides were cleaved from the resin with HF thereby providing both the "binding" and "coding" sequences as free peptides. The amino acid composition, mass spectro-scopy and N-terminal sequencing data are consistent with the correct products. (See Figures 5, 6 and 7.)

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Mass Spectrometry:

	theoretical	observed
Ddz peptide	580.2	580.2
Fmoc peptide	666.9	666.4

Amino Acid Composition:

Fmoc Peptide			Ddz Peptide		
	Theor. (%)	Observ. (%)		Theor. (%)	Observ. (%)
His	20	21	Ala	33	33
Thr	20	20	Glu	17	17
Val	20	19	Phe	17	17
Leu	20	29	Gly	17	15
Arg	20	20	Ser	17	15

N-Terminal Edman Sequencing of Resin beads (coding peptide only):

Cycle #	amino acid	pmol
1	Ala	45
2	Ser	12
3	Gly	30
4	Glu	28
5	Phe	28
6	Ala	29

Example 2

Synthesis of a Solution-Phase Peptide Library Model

In this example, a 1:1 solution-phase adduct between a "binding" and a "coding" strand was synthesized and fully characterized. The "binding" strand was assembled with Fmoc-protected monomers, and the "coding" strand was assembled with Ddz-protected monomers.

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"Binding" sequence: Ac-Glu-Ser-Thr-Arg-Pro-nLeu-Lys-ß-
(Fmoc peptide)   Ala-NH₂

"Coding" sequence: H₂N-Gly-Ala-Phe-Gly-Ala-Phe-CONH
(Ddz peptide)

Synthesis Scheme:

Step #	Description
1	Derivatization of Rink Resin with Fmoc-B-Ala-OH spacer
2	Fmoc deprotection with 20% piperidine/DMF
3	Coupling of Fmoc-Lys (4-methoxybenzyloxycarbonyl)-OH
4	Fmoc deprotection of "binding" strand with 20% piperidine/DMF
5	Coupling of first "binding" monomer Fmoc-nLeu-OH
6	Cleavage of "coding" strand 4-Methoxybenzyloxycarbonyl (Moz) group with 7.5% TFA/CH₂Cl₂
7	Coupling of first "coding" amino acid Ddz-Phe-OH
8	Repeat steps 4-7 with corresponding amino acids
9	Final Fmoc deprotection and acetylation
10	Cleavage and deprotection of resin sample with TFA

Following TFA cleavage and deprotection, the model solution-phase library contains a 1:1 Fmoc/Ddz conjugate peptide. One peptide sequence was synthesized and not a mixture in order to fully characterize the reaction product. The amino acid composition and mass spectroscopy data are consistent with the correct product. In addition, the "binding" and "coding" hybrid peptides were tested in a competition ELISA format. The ELSTRPnL "binding" sequence binds to an anti-gp120 antibody with submicromolar affinity. This value was not affected by the presence of the "coding" peptide.

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Mass Spectroscopy:

	Theoretical	Observed
Fmoc/Ddz peptide conjugate:	1492.7	1492.6

Amino Acid Composition:

Fmoc Peptide			Ddz Peptide		
	Theor. (%)	Observ. (%)		Theor. (%)	Observ. (%)
Glu	7.1	7.0	Phe	4.2	14.7
Ser	7.1	7.1	Ala	14.2	14.4
Thr	7.1	7.2	Gly	14.2	13.8
Arg	7.1	7.2			
Pro	7.1	7.9			
Nleu	7.1	6.3			

The instant invention is shown and described herein in what is considered to be the most practical, and preferred embodiments. It is recognized, however, that departures may be made therefrom which are within the scope of the invention, and that obvious modifications will occur to one skilled in the art upon reading this disclosure.

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CLAIMS

1. An assay conjugate, comprising:
an active polymer comprising monomers
selected from the group consisting of peptide and
peptoid monomers;
an encoding polymer comprising encoding
monomers, wherein the encoding polymer corresponds to
and allows identification of the active polymer; and
a coupling moiety covalently coupled to the
active peptide and the encoding polymer.

2. The conjugate of claim 1, wherein the
coupling moiety comprises a solid support.

3. The conjugate of claim 1, wherein the
coupling moiety comprises a soluble linking group.

4. The conjugate of claim 1, wherein all
active polymers coupled to a single selected solid
support are identical.

5. The conjugate of claim 1, wherein the
active polymer comprises a polypeptide and the
encoding polymer comprises a DNA or RNA oligonucleo-
tide.

6. The conjugate of claim 1, wherein the
active polymer comprises a polypeptoid and the
encoding polymer comprises a DNA or RNA oligonucleo-
tide.

7.   The conjugate of claim 6, wherein the
polypeptoid comprises a polymer of monomers of the
formula:

5

10

wherein R is alkyl of 2-6 carbon atoms, haloalkyl of
1-6 carbon atoms wherein halo is F, Cl, Br, or I,
alkenyl of 2-6 carbon atoms, alkynyl of 2-6 carbon
15   atoms, cyclolkyl of 3-8 carbon atoms, alkoxyalkyl of
2-8 carbon atoms, aryl of 6-10 carbon atoms, arylalkyl
of 7-12 carbon atoms, arylalkyl of 7-12 carbon atoms
substituted with 1-3 radicals independently selected
from halo and nitro and hydroxy, aminoalkyl of 1-6
20   carbon atoms, hydroxyalkyl of 1-6 carbon atoms,
carboxy, carboxyalkyl of 2-6 carbon atoms,
carboalkoxy-alkyl of 3-10 carbon atoms, carbamyl,
carbamylalkyl of 2-6 carbon atoms, imidazolyl, imid-
azolylalkyl of 4-10 carbon atoms, pyridyl,
25   pyridylalkyl of 6-10 carbon atoms, piperidyl, pip-
eridylalkyl of 5-10 carbon atoms, indolyl, or
indolyalkyl of 9-15 carbon atoms.

8.   A mixture of conjugates of claim 1,
30   wherein the mixture comprises at least two distinct
active polymers, the coupling moiety is a solid
support, distinct active polymers are covalently
coupled to separate solid supports and a distinct
encoding polymer corresponding to each active polymer
35   is covalently coupled to the support coupled to its
corresponding active polymer.

9.   The mixture of claim 8, wherein the mixture comprises at least ten distinct active polymers.

5   10.   The mixture of claim 9, wherein the mixture comprises at least 100 distinct active polymers.

11.   A conjugate, comprising:
10   a biologically active peptide comprised of five or more amino acids;
a encoding polymer comprised of nucleic acids wherein one or more nucleic acids within the encoding polymer correspond to and can be readily
15   identified with the amino acids of the active polymer; and
a coupling moiety covalently coupled to the active peptide and the encoding polymer.

20   12.   The conjugate of claim 11, wherein the amino acids are selected from the group consisting of alanine, cysteine, aspartic acid, glutamic acid, phenylalanine, glycine, histidine, isoleucine, lysine, leucine, methionine, asparagine, proline, glutamine,
25   arginine, serine, threonine, valine, tryptophan, and tyrosine.

13.   The conjugate of claim 11, wherein the encoding polymer includes three nucleotides for each
30   amino acid of the active polymer and the three nucleotides are nucleotides which naturally encode the corresponding amino acid of the active polymer.

14.   The conjugate of claim 11, wherein the
35   coupling moiety comprises a solid support.

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15.   The conjugate of claim 14, wherein the
solid support is in the form of a spherical bead
having a diameter of less than one centimeter.

5   16.   The conjugate of claim 11, wherein the
coupling moiety comprises a soluble linking group.

17.   A method of synthesizing an encoded
peptide or peptoid polymer, the method comprising:
10   a)   providing a coupling moiety;
           b)   coupling to the coupling moiety an
                 active monomer, and coupling to the
                 coupling moiety an encoding monomer
                 which corresponds to the active monomer
15   to form a conjugate having a bound
                 active monomer and a bound encoding
                 monomer; and
           c)   repeating step b) until an active
                 polymer of the desired length is
20   obtained.

18.   The method of claim 17 wherein the
active monomers are amino acids and the encoding
monomers are nucleic acid bases.
25
19.   A method of synthesizing a mixture of
encoded polymers, the method comprising:
           a)   providing a plurality of coupling
                 moieties;
30   b)   dividing the plurality of coupling
                 moieties into a plurality of aliquots;
           c)   within each aliquot, coupling to each
                 coupling moiety an active monomer, and
                 coupling to each said coupling moiety
35   an encoding monomer which corresponds
                 to the active monomer to form a
                 plurality of conjugates having a bound
                 active monomer and a bound encoding

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monomer, each in a separate aliquot, wherein different aliquots may contain different active monomers;

d) combining the aliquots of conjugates to form a mixture of conjugates; and

e) repeating steps b-d) until a mixture of active polymers of the desired length is obtained.

20. The method of claim 19, wherein the active monomers comprise activated amino acids and the coupling moiety comprises a solid support.
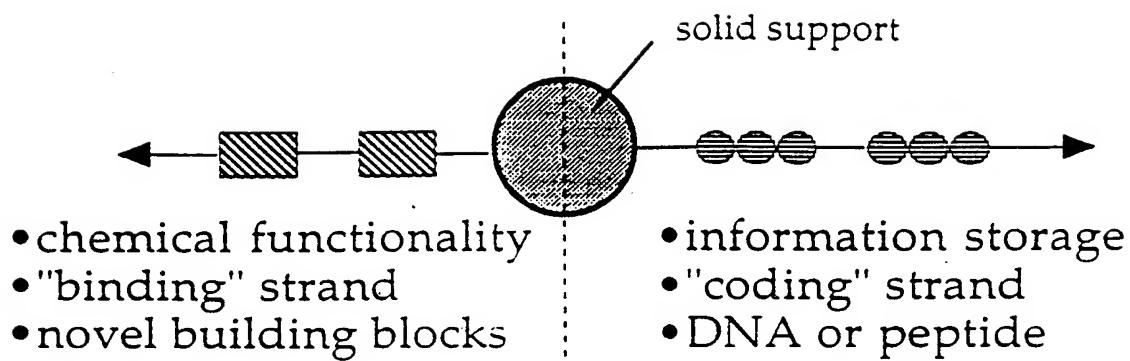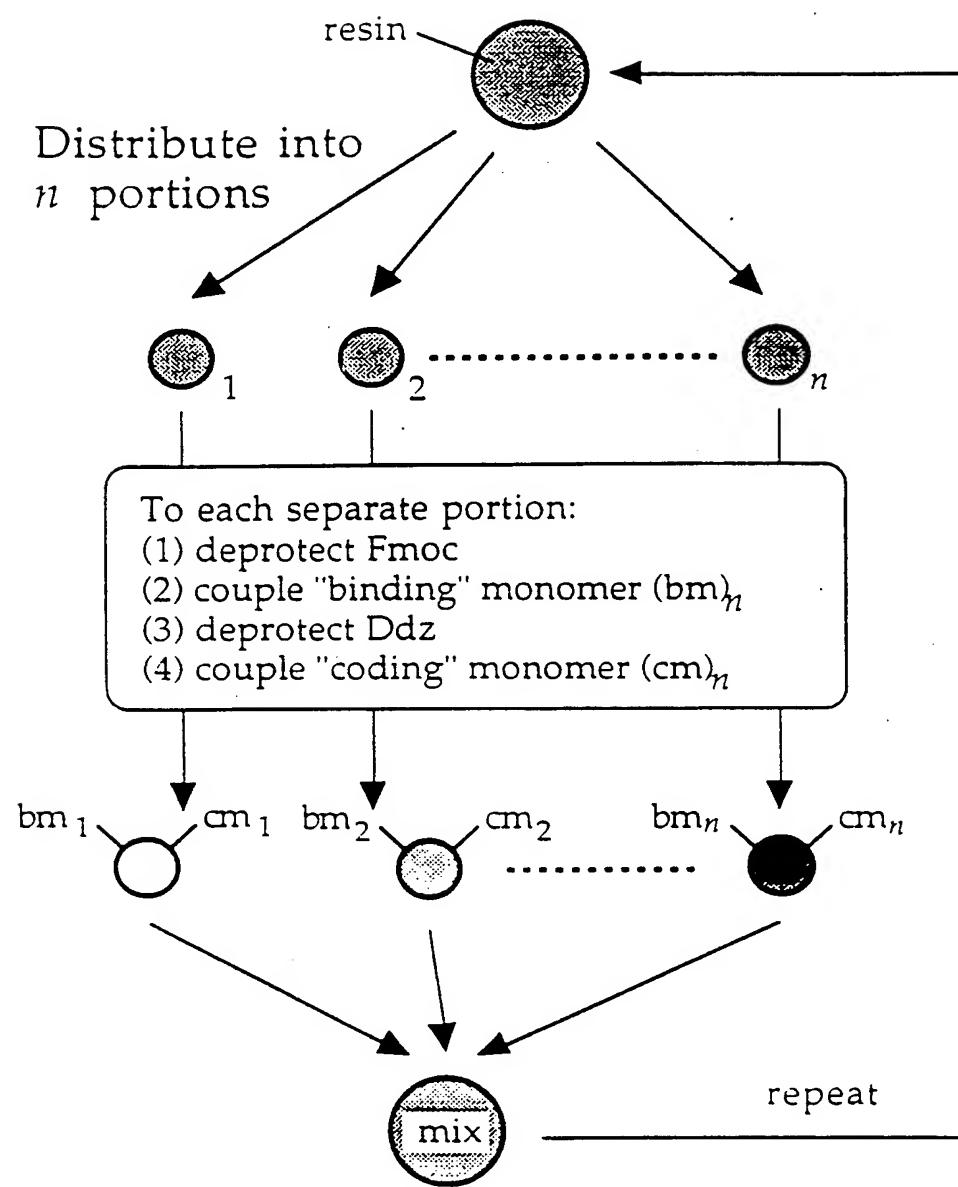
FIGURE 1

FIGURE 2

(1) Resin-bound:

polymer/DNA   Fmoc-NH ⌁⌁⌁ ● ⌁⌁⌁ O-DMT

polymer/peptide   Fmoc-NH ⌁⌁⌁ ● ⌁⌁⌁ NH-Ddz

(2) Solution-phase conjugate:

polymer/DNA   polymer/peptide

Fmoc-NH O-DMT   Fmoc-NH NH-Moz
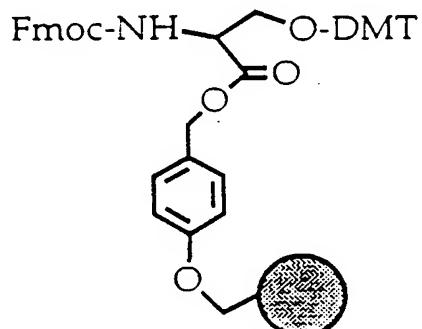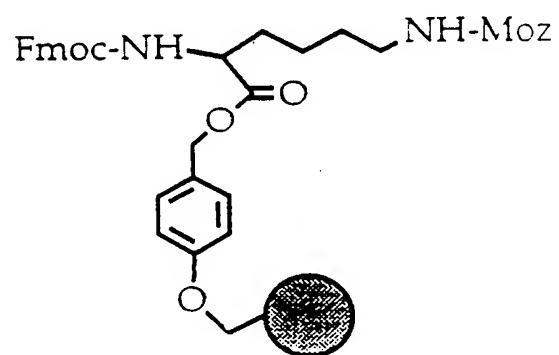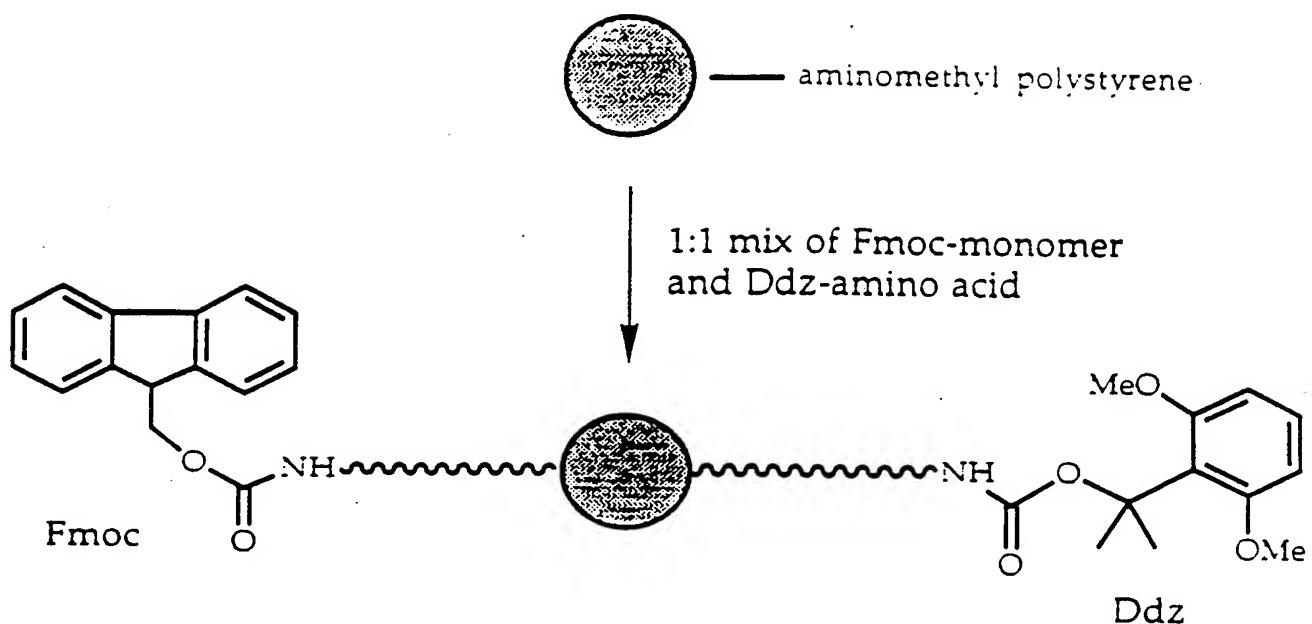
FIGURE 3

FIGURE 4

HPLC chromatogram of HF cleaved/deprotected resin sample:
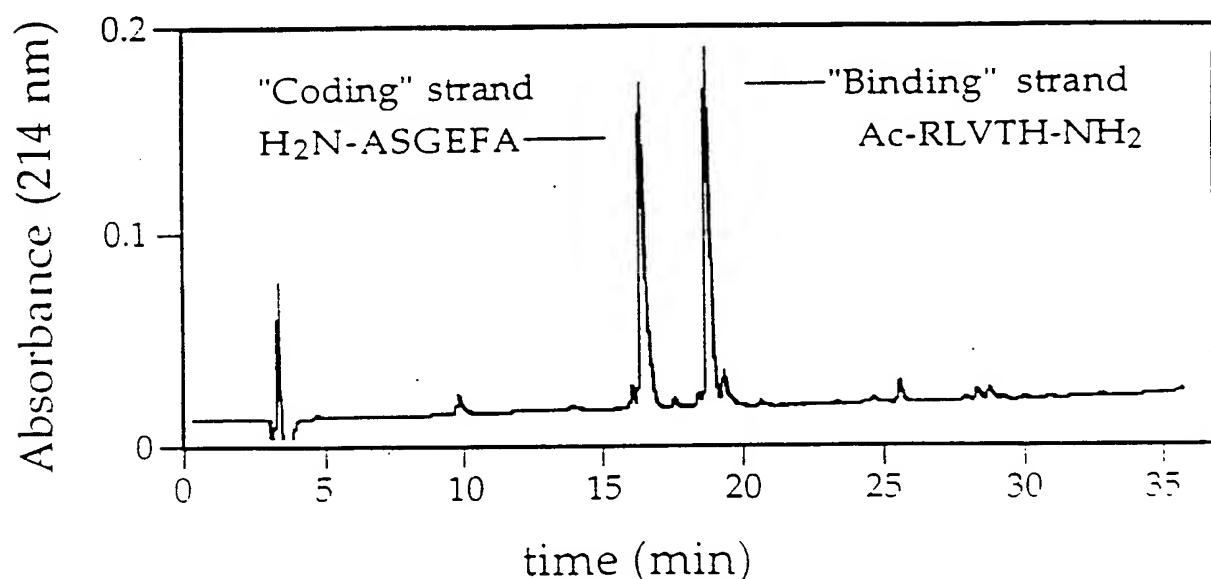
FIGURE 5

HPLC chromatogram of TFA cleaved / deprotected peptide:
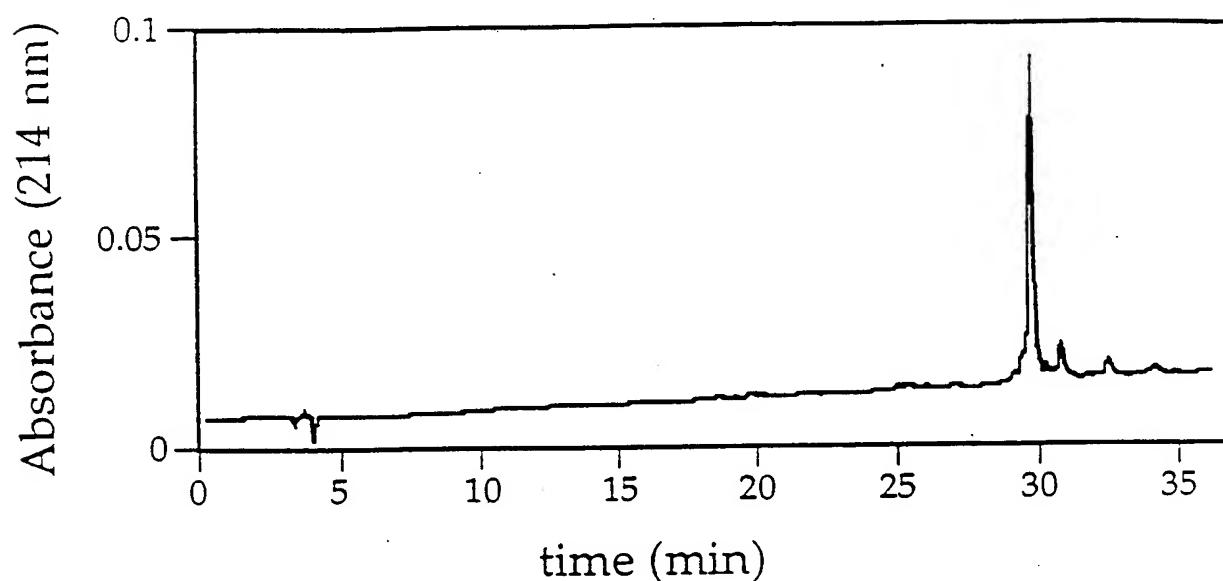

FIGURE 6

Competition ELISA of "binding" sequence vs. "binding/coding" sequence
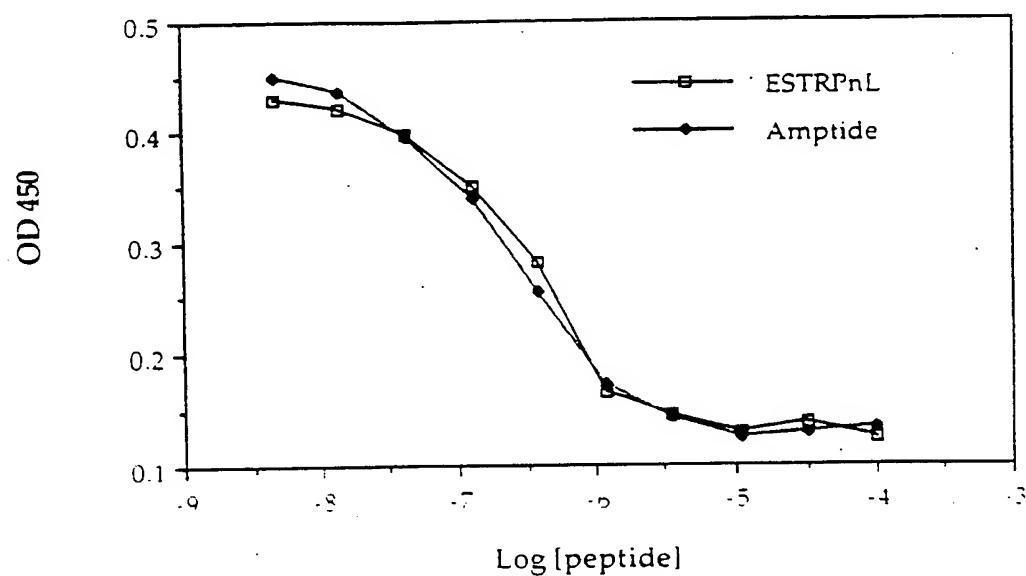
FIGURE 7

Analysis of solid-phase Amptides

• simultaneous synthesis of peptide & corresponding oligo
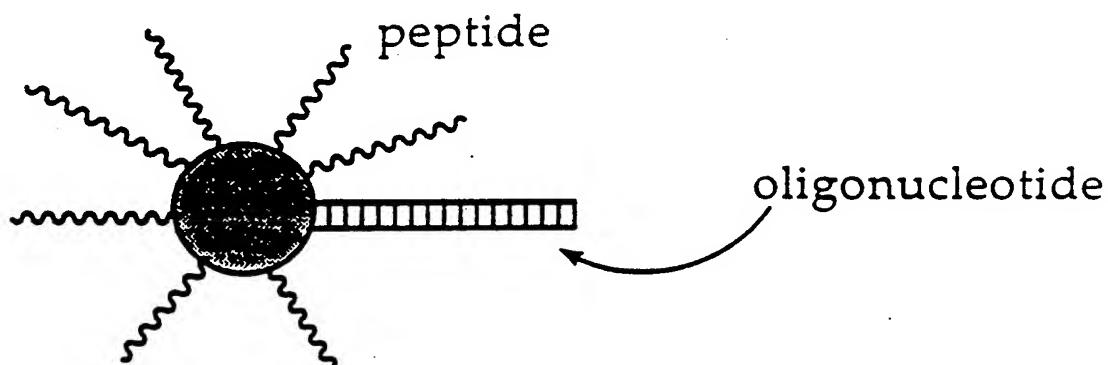
• detection/isolation of bead of interest
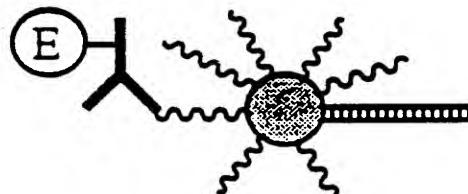


• DNA sequencing/amplification directly on a solid-phase*
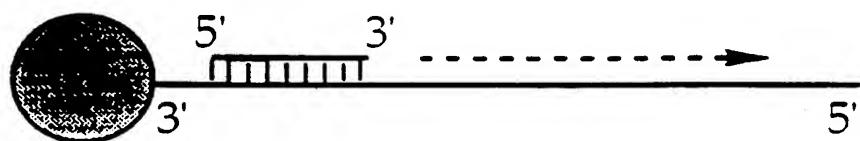

FIGURE 8

Analysis of solution-phase amptides
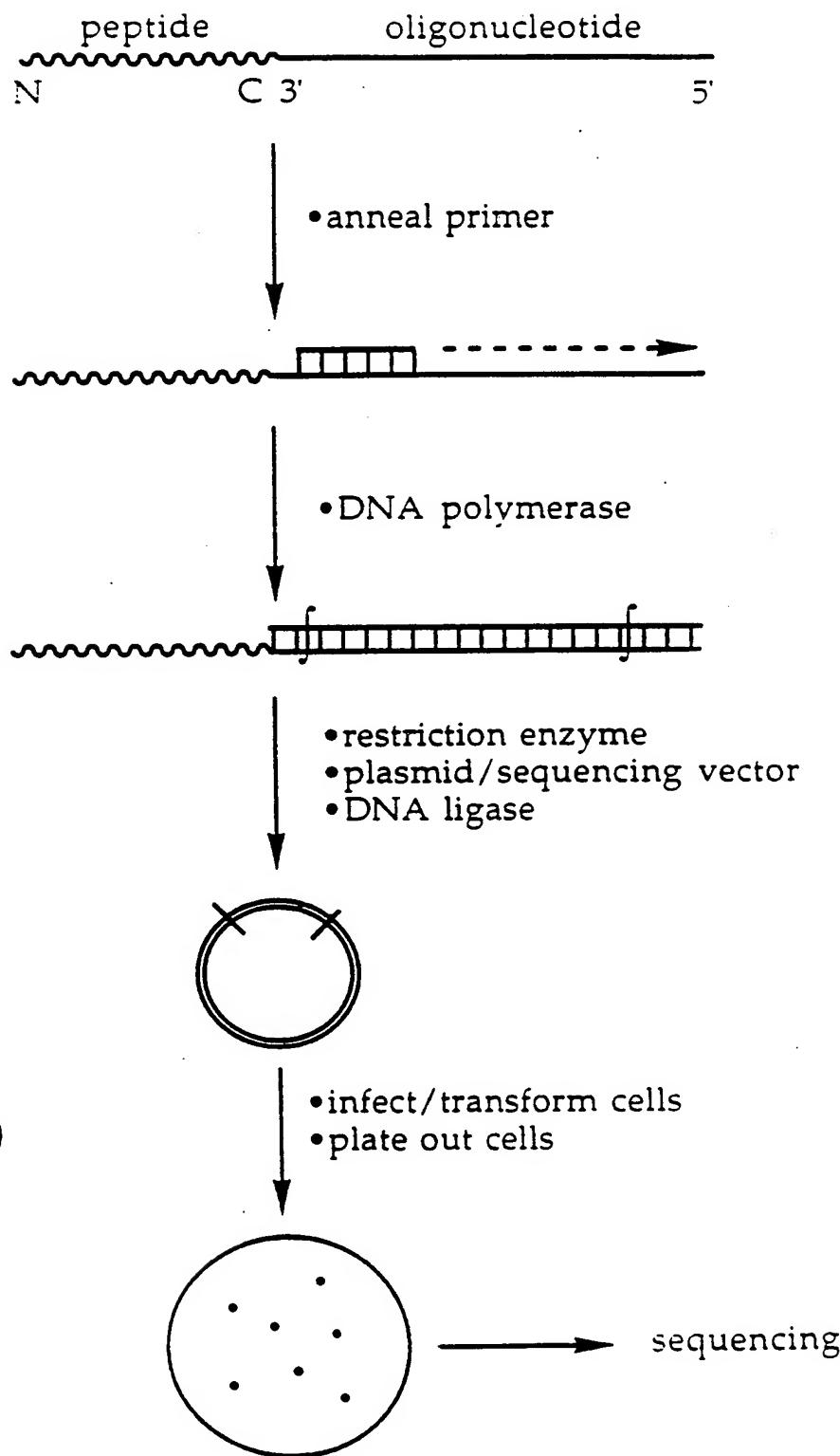
FIGURE 9

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C07C 237/00; C07H 21/00; C07K 1/00, 5/00, 7/00, 13/00, 17/00
US CL :530/300, 350, 333, 334, 338, 402; 536/25.3; 564/159

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/300, 350, 333, 334, 338, 402; 536/25.3; 564/159

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG
search terms: oligonucleotide, DNA, peptide, synthesis, combinatorial

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	Proceedings of the National Academy of Sciences U.S.A., Vol. 89, issued June 1992, S. Brenner et al., "Encoded combinatorial chemistry", pages 5381-5383, see the entire document.	1, 2, 4, 5, 8-12, 14, 15, 17-20 ---------- 3, 6, 7, 13, 16
X --- Y	Science, Vol. 257, issued 17 July 1992, I. Amato, "Speeding up a chemical game of chance", pages 330-331, see the entire document.	1, 2, 4, 5, 8-12, 14, 15, 17-20 ---------- 3, 6, 7, 13, 16

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be part of particular relevance	
"E" earlier document published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
01 February 1994	24 FEB 1994

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	ERIC GRIMES
Facsimile No. NOT APPLICABLE	Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P ---- Y, P	Proceedings of the National Academy of Sciences U.S.A., Vol. 90, issued November 1993, M. C. Needels et al., "Generation and screening of an oligonucleotide-encoded synthetic peptide library", pages 10700-10704, see the entire document.	1, 2, 4, 5, 8-12, 14, 15, 17-20 ----------- 3, 6, 7, 13, 16
Y	Science, Vol. 254, issued 06 December 1991, P. E. Nielsen et al., "Sequence-selective recognition of DNA by strand displacement with a thymine-substituted polyamide", pages 1497-1500, see page 1498.	6, 7
Y	BioTechniques, Vol. 13, No. 3, issued September 1992, R. A. Houghten et al., "The use of synthetic peptide combinatorial libraries for the identification of bioactive peptides", pages 412-421, see the entire document.	1-20
Y	US, A, 4,359,353 (KYDD) 16 November 1982, see columns 2-3.	1-20
A	Nature, Vol. 354, issued 07 November 1991, K. S. Lam et al., "A new type of synthetic peptide library for identifying ligand-binding activity", pages 82-84.	1-20
A	Science, Vol. 249, issued 27 July 1990, J. J. Devlin et al., "Random peptide libraries: a source of specific protein binding molecules", pages 404-406.	1-20